

Amendment and Response

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Serial No.: 09/772,598

Confirmation No.: 2967

Filed: January 30, 2001

For: CRYSTALLIZATION AND STRUCTURE DETERMINATION OF STAPHYLOCOCCUS AUREUS NAD SYNTHETASE

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1-34. (Canceled)

35. (Previously Presented) A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:

providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

crystallizing *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO).

36-37. (Canceled)

38. (Previously Presented) A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase.

39. (Currently Amended) A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase having the monoclinic ~~trigonal~~ space group symmetry $P2_1$.

40. (Previously Presented) A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising a unit cell having dimensions of a, b, and c; wherein a is about 40Å to about 60Å, b is about 90Å to about 120Å, and c is about 80Å to about 110Å; and wherein $\alpha = \gamma = 90^\circ$ and β is about 80° to about 120°.

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41. **(Previously Presented)** A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 1.
42. **(Previously Presented)** A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase having amino acid sequence SEQ ID NO:1.
43. **(Previously Presented)** A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase having amino acid sequence SEQ ID NO:1, with the proviso that at least one methionine is replaced with selenomethionine.
44. **(New)** A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:
- providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and
 - forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),
 - wherein the crystal has the monoclinic space group symmetry P2₁.
45. **(New)** The method of claim 44 wherein the solution comprises 18% by weight to 22% by weight PEG 1500.
46. **(New)** A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:
- providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

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forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),

wherein the crystal comprises a unit cell having dimensions of a, b, and c; wherein a is about 40Å to about 60Å, b is about 90Å to about 120Å, and c is about 80Å to about 110Å; and wherein $\alpha = \gamma = 90^\circ$ and β is about 80° to about 120° .

47. (New) The method of claim 46 wherein the solution comprises 18% by weight to 22% by weight PEG 1500.

48. (New) A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:

providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),

wherein the crystal comprises atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 1.

49. (New) The method of claim 48 wherein the solution comprises 18% by weight to 22% by weight PEG 1500.

50. (New) A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:

providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

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forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),

wherein the crystal of *S. aureus* NAD synthetase has an *S. aureus* NAD synthetase amino acid sequence SEQ ID NO:1.

51. (New) The method of claim 50 wherein the solution comprises 18% by weight to 22% by weight PEG 1500.

52. (New) A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:

providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),

wherein the crystal of *S. aureus* NAD synthetase has an *S. aureus* NAD synthetase amino acid sequence SEQ ID NO:1, except that at least one methionine is replaced with selenomethionine.

53. (New) The method of claim 52 wherein the solution comprises 18% by weight to 22% by weight PEG 1500.

54. (New) A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase, wherein the crystal effectively diffracts x-rays to a resolution of 1.5Å to 3Å.

55. (New) The crystal of claim 54 wherein the resolution is at least 2.2Å.

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56. (New) A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:

providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),

wherein the crystal effectively diffracts x-rays to a resolution of 1.5Å to 3Å.

57. (New) The method of claim 56 wherein the resolution is at least 2.2Å.

58. (New) A crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase, wherein the crystal has at least one dimension of 0.15-0.8 mm.

59. (New) The crystal of claim 58 having dimensions of 0.15-0.8 mm x 0.2 mm x 0.05-0.1 mm.

60. (New) The crystal of claim 58 wherein the crystal effectively diffracts x-rays to a resolution of 1.5Å to 3Å.

61. (New) The crystal of claim 60 wherein the resolution is at least 2.2Å.

62. (New) A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:

providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

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forming a crystal of *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO),

wherein the crystal has at least one dimension of 0.15-0.8 mm.

63. (New) The method of claim 62 wherein the crystal has dimensions of 0.15-0.8 mm x 0.2 mm x 0.05-0.1 mm.

64. (New) The method of claim 62 wherein the crystal effectively diffracts x-rays to a resolution of 1.5Å to 3Å.

65. (New) The method of claim 64 wherein the resolution is at least 2.2Å.

66. (New) The method of claim 35 wherein the solution comprises 18% by weight to 22% by weight PEG 1500.